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EFFECTS OF THE DOUGLAS-FIR TUSSOCK MOTH NUCLEOPOLYHEDROSIS VIRUS (BACULOVIRUS) ON THREE SPECIES OF SALMONID FISH

G.M. Banowetz
J.L. Fryer
P.J. Iwai
M.E. Martignoni

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Pacific Northwest Forest and Range Experiment Station
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AUTHORS:

G. M. Banowetz and J. L. Fryer are with the Department of Microbiology, Oregon State University, Corvallis.

P. J. Iwai and M. E. Martignoni are with the USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Corvallis, Oregon.

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Reference Abstract

Banowetz, G. M., J. L. Fryer, P. J. Iwai, and M. E. Martignoni.
1976. Effects of the Douglas-fir tussock moth nucleopolyhedrosis virus (*Baculovirus*) on three species of salmonid fish. USDA For. Serv. Res. Pap. PNW-214, 6 p. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and steelhead trout (*Salmo gairdneri*) showed no pathology when exposed to *Baculovirus* by three different routes. Cell lines derived from chinook salmon and steelhead trout were refractory to nonoccluded virions, and no cytopathology was observed by light and electron microscopy. Both polyhedra and nonoccluded virions were inactivated rapidly by coho salmon exposed to the virus by three different routes.

KEYWORDS: Virus (insect) (-salmonids, nucleopolyhedrosis, *Baculovirus*, Douglas-fir tussock moth, *Orgyia pseudotsugata*.

RESEARCH SUMMARY

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This study examined whether the use of a nucleopolyhedrosis virus from *Orgyia pseudotsugata* for control of Douglas-fir tussock moth infestations would be harmful to certain salmonid fish residing in waters adjacent to the affected forests.

The virus caused no pathology in two salmonid cell lines or in three species of salmonid fish. Furthermore, the virus survived less than 24 hours in coho salmon exposed to the agent by the waterborne route, feeding, or injection. It also appeared that coho salmon might not eat *O. pseudotsugata* larvae when other food was available.

Contents

Introduction

Douglas-fir tussock moth (*Orgyia pseudotsugata*) infestations of forests in western North America have caused severe economic losses to the forest industry (Wickman et al. 1973). As recently as 1973, an outbreak occurred in northeast Oregon and southeast Washington which affected nearly 500,000 acres and resulted in extensive defoliation of economically important species of fir trees. In the past, chemical pesticides were used to control moth outbreaks; however, due to certain problems associated with the use of these pesticides, other control measures became desirable.

Two nucleopolyhedrosis viruses belonging to subgroup A of the genus *Baculovirus* have been isolated from *O. pseudotsugata* (Hughes and Addison 1970). Both viruses occur naturally and are major factors in terminating moth infestations. One of these, designated BV (bundle virus), is characterized by bundles of nucleocapsids common to a single envelope. This virus has been registered by the U.S. Environmental Protection Agency for use as a biological control agent against the larvae (Registration No. 27586-1, August 11, 1976). The information presented here was needed to demonstrate safety of the viral agent for fish.

This study investigated the effects of BV on economically important species of salmonid fishes which may reside in waters within forest areas subject to tussock moth infestation. Experiments were conducted to observe whether the virus affected either live fish or fish cell lines and to determine if BV persisted in the animals when introduced by three different routes.

Materials and Methods

VIRUS PREPARATIONS

A suspension of nonoccluded virions was prepared as follows: hemolymph from 25 larvae (average weight 300 mg), inoculated per os with pure BV inclusion bodies 5 days prior to the bleeding, was collected into a sterile conical centrifuge tube in an ice bath (Martignoni and Scallion 1961). Sufficient tissue culture medium was added such that the hemolymph was diluted 1:40. Hereafter, the tissue culture medium (Auto-Pow, Flow Laboratories, Inc.)[1/] supplemented with streptomycin (100 µg/ml), penicillin (100 IU/ml), and 10 percent fetal bovine serum will be referred to as MEM-10 percent. The hemolymph suspension was then passed through a 0.45-µm filter and stored at 4 °C. A 1:1000 dilution of this preparation was used for all the in vivo experiments requiring nonoccluded virions.

A second suspension of BV virions was prepared and used for the in vitro experiments with cell lines. This preparation was diluted 1:10 with MEM-10 percent and stored at 4 °C.

A commercial preparation of BV polyhedra (Nutrilite 1973, Lot 1), was used for in vivo experiments requiring the occluded virions. This also was stored at 4 °C.

INSECTS

Inbred strain GL *Orgyia pseudotsugata* larvae were used throughout this study. These animals were fed an artificial diet and maintained at 22 °C in 100- x 15-mm disposable petri dishes. Second-instar, 75-mg and 300-mg larvae were used for specific tests in this investigation.

SALMONID CELL LINES

Two salmonid cell lines were employed in this study. They were CHSE-214, derived from chinook salmon (*Oncorhynchus tshawytscha*) embryos, and STE-137, derived from steelhead trout (*Salmo gairdneri*) embryos (Fryer et al. 1965).

[1/] Trade names used should not be construed as endorsement by Department of Agriculture.

The cells were maintained at 18 °C in MEM-10 percent throughout this study. At the time of these experiments, the CHSE-214 cells had been subcultured 225 times and the STE-137 cells 159 times.

FISH

Chinook salmon fingerlings (average weight 0.5 g) were obtained from the Leaburg Trout Hatchery (Oregon Department of Fish and Wildlife). Coho salmon (*O. kisutch*) fingerlings (average weight 0.5 g) and smolts (average weight 20 g) were supplied by the Alsea Salmon Hatchery (Oregon Department of Fish and Wildlife). Steelhead trout were obtained from the Nehalem River Salmon Hatchery (Oregon Department of Fish and Wildlife).

All fish were maintained in constantly flowing 13 °C well water at the Oregon State University Fish Disease Laboratory and fed Oregon Moist Pellets. The well water at this laboratory contained no fish pathogens.

BIOASSAY PROCEDURES

Intrahemocoelic injection of 75-mg larvae was used to detect and quantitate nonoccluded BV virions. Larvae were anesthetized with ether, disinfected in 0.4 percent Hyamine, and injected in the second proleg with 5.0 µl of virus suspension. A calibrated Agla micrometer syringe (Burroughs Wellcome Co.) fitted with a Tomac 0.25-ml tuberculin syringe and disposable 30-gage 13-mm needle (B-D Yale) was used for all injections. The injected larvae were maintained at 30 °C for 8 days after the inoculation. All dead larvae were examined microscopically for polyhedra in the tracheal matrix near the midgut. Larvae with polyhedra in this region were recorded as deaths attributable to BV.

A peroral bioassay involving addition of virus to the surface of the ration[2/] was used to detect and quantitate BV polyhedra. Disposable analyzer cups (2 ml) were filled to the 1-ml level with warm diet, then allowed to airdry for 1-2 hours (h). Each cup then received 20 µl of the virus-containing suspension so that the entire diet surface was wetted. After the diet surface had dried, a second-instar larva was placed in each cup, a cap perforated for air exchange was positioned, and the cups were incubated at 30 °C for 12 days. Only larvae which had molted to the second instar within 18 h before the test were used. Dead larvae were examined for polyhedra as described above.

When quantitation of BV activity was desired, replicate groups of 20 larvae were used for each of the bioassay procedures. If the test was employed only to detect the presence of BV, one group of 20 larvae was used for each sample. The mortality data from intrahemocoelic assays were evaluated by means of logit chi-square analysis (Berkson 1953). We used the LOCSAN program by R. L. Giese, Purdue University. LD50 determined by intrahemocoelic injection are designated IH-LD50.

EXPOSURE OF FISH CELL LINES TO BACULOVIRUS

Cell cultures (CHSE-214 and STE-137) were propagated at 18 °C in 32-ounce prescription bottles to 80-90 percent monolayer before being exposed to BV. At that point, the medium was removed from the cells and replaced with MEM-10 percent which contained BV nonoccluded virions (9.90×10^5 IH-LD50 units/ml). After 24-h exposure to the virus, the medium was again removed from the bottles. A portion of the cells was used for electron microscopic examination while the remainder was subcultured. The virus-containing medium was bioassayed after it was removed from the cell lines. Cells exposed to virus were transferred through five successive generations

2/ Iwai, Paul J., and Mauro E. Martignoni. 1976. Peroral bioassay of activity of *Baculovirus* of *Orgyia pseudotsugata*: Exposure by diet-surface treatment (DST). 11 p. Unpublished laboratory procedure on file at the Forestry Sciences Laboratory, Corvallis, Oregon.

at 7- to 10-day intervals and examined by light microscopy for any pathological changes.

Cells to be examined by electron microscopy were fixed with 2.5 percent glutaraldehyde in Sørensen's phosphate buffer at pH 7.0 followed by 1 percent osmium tetroxide. The samples were dehydrated in acetone and embedded in an Epon-Araldite mixture. Preparative work and electron microscopy were done by A. H. Soeldner, Department of Botany and Plant Pathology, Oregon State University. The samples were examined with a Phillips 300 electron microscope at a magnification of 30,000.

EXPOSURE OF SALMONID FINGERLINGS TO BACULOVIRUS

The animals used for these experiments were of a size and age considered most likely to show any virus-induced pathologies. These fish were exposed to BV by three different routes; a waterborne exposure, intraperitoneal (ip) injection, and by feeding.

The waterborne exposure was conducted as follows: 200 animals were placed in 4 gallons of aerated water which was constantly agitated with a magnetic stirring bar. Sufficient polyhedra were added to the water to give an effective dose of 100-surface-acre doses of virus, adjusted to the surface area of the container employed. After 18-h exposure, the animals were transferred to 13 ^{o}C water and maintained and observed there for 30 days. At that time, a representative sample of fish was placed in Bouin's fixative and submitted to Dr. R. Stroud, Department of Veterinary Science, Oregon State University, for histopathological examination.

Fish were injected with BV nonoccluded virions in the following manner: the animals were anesthetized, then injected ip with 20 µl of a suspension which contained 1.67 x 10^2 IH-LD50 units of BV virus. The fish were maintained in 13 ^{o}C water for 30 days. At that time a sample of animals was submitted for histopathological examination.

The 200 animals exposed by the oral route were fed polyhedra incorporated into their food at a rate equivalent to 100-acre doses adjusted to the surface area of the tanks. The fish consumed this amount of food within a period of 24 h. After 30 days had elapsed, a sample of these fish was submitted for histopathological examination.

Coho salmon, chinook salmon, and steelhead trout were exposed to BV by each route described above. Control animals of each species were also maintained in 13 ^{o}C well water and submitted for histopathological examination at the same time the exposed animals were collected.

SURVIVAL OF BACULOVIRUS *IN COHO SALMON SMOLTS*

The purpose of these experiments was to determine whether BV persisted in the fish and, if so, in which organ(s) it resided. Smolts were chosen for this work because their size allowed examination of individual internal organs. These animals also were exposed to the virus by the waterborne route, ip injection, and the oral route. Each experiment was done with triplicate groups of fish.

The procedure used for the waterborne exposure was identical to that described for fingerlings except that only 15 animals were held in the virus-containing water. The water was bioassayed for polyhedra at the start of the experiment and after the 18-h exposure period. With time 0 defined as the end of the 18-h exposure period, fish were sampled at time 0, 12, 24, 48, and 96 h. At each sampling time, three fish were sacrificed. The pooled kidneys, livers, and spleens from each sample group were weighed, diluted 1:10 with MEM-10 percent, ground in a Virtis tissue grinder, and assayed for polyhedra. Pooled intestines from these fish were treated identically.

Fish which received ip injections of BV nonoccluded virions were anesthetized and then injected with 0.1 ml of a suspension containing 8.33×10^2 tussock moth IH-LD50 units. Sampling began 8 h later with additional samples at 24, 48, and 96 h. At each sampling time, three fish were sacrificed. Kidneys and spleens were removed and pooled. A separate sample of liver tissues was treated identically. Each sample was weighed, diluted 1:10 with MEM-10 percent, ground in a Virtis tissue grinder, centrifuged at 1000 g for 15 minutes, and passed through a 0.45-mm filter. The samples then were assayed for the presence of BV.

Initially, the oral route exposure was to be via infected larvae. The salmon (even when starved up to 5 days) would not eat larvae. Even larvae coated with 10 percent bovine serum albumin to induce sinking were refused. Thus, these smolts received an oral exposure to BV by the addition of polyhedra to their food. As with the fingerlings, the equivalent of 100-surface-acre doses, adjusted to the surface area of the tanks, were fed within a period of 24 h. After the fish were fed the polyhedra, they were sampled at time 0, 4, 8, 24, and 48 h. Sampling was done in the following manner: at each sampling time, three animals were sacrificed and their digestive tracts were removed and pooled. The tissue was weighed, diluted 1:10 with MEM-10 percent, and homogenized in a Virtis tissue grinder. The resulting homogenate was then assayed for the presence of polyhedra.

Results

EFFECT OF BACULOVIRUS *ON SALMONID CELL LINES*

No pathological changes were observed in either cell line (CHSE-214 and STE-137) exposed to BV virions. The cells exposed to the virus appeared identical to control cells when examined by either light or electron microscopy. No change occurred in the growth rate of the cells or in their response to subculture. K. Hughes (Forestry Sciences Laboratory, Corvallis, Oregon) also examined the grids prepared for electron microscopy and saw no evidence that the virus had entered into or altered the cells.

EFFECT OF BACULOVIRUS *ON SALMONID FINGERLINGS*

Coho salmon, chinook salmon, and steelhead trout fingerlings were not adversely affected by exposure to BV by a any of the three routes. The exposed animals appeared healthy and showed growth similar to the control fish through the period of the experiment. No histopathology attributable to BV was observed in any of the exposed animals, and no virus was recovered from fish sampled 30 days after exposure to BV.

SURVIVAL OF BACULOVIRUS *IN COHO SALMON SMOLTS*

Both polyhedra and BV virions were inactivated rapidly by the salmon (table 1). Within 24 h after the exposure period, the virus was inactivated regardless of the route of exposure. The animals were particularly refractory to polyhedra added to the water supply.

In addition to the insusceptibility of the fish to this virus, it also appeared that fish would not likely eat *O. pseudotsugata* larvae. Three groups of fish, deprived of food for 5 days prior to the experiment rejected larvae while they did not reject earthworms cut to the same size as the larvae.

Discussion

This study indicates that the use of BV as an agent to control *O. pseudotsugata* infestations should have no deleterious effects on chinook salmon, coho salmon, or steelhead trout which reside in waters adjacent to treated forests. Coho salmon seem reluctant to eat *O. pseudotsugata*

Table 1--*Survival of a Douglas-fir tussock moth* (Orgyia pseudotsugata) *nucleopolyhedrosis virus* (Baculovirus) *in coho salmon* (Oncorhynchus kisutch) *smolts when administered by three different routes*

Exposure route	Organ(s) sampled	Virus recovery at postexposure times			
		8 h	24 h	48 h	96 h
Intraperitoneal[a] injection	Kidney, spleen	+[d]	--	--	--
	Liver	+[d]	--	--	--
Waterborne[b]	Kidney, liver, spleen	-[d]	--	--	--
	Digestive tract	-[e]	--	--	--
Oral[c]	Digestive tract	+[e]	--	--	--

[a]Salmon were injected with 8.33×10^2 *O. pseudotsugata* larvae IH-LD50 units of nonoccluded virions.

[b]The equivalent of 100 surface acre doses (10^{13} polyhedra/acre), adjusted to the surface area of fish tank, were added to the water and fish exposed for 18 h.

[c]The equivalent of 100 surface acre doses (10^{13} polyhedra/acre), adjusted to the surface area of fish tank, were fed within a period of 24 h.

[d]Virus recovery by intrahemocoelic injection in 75 mg *O. pseudotsugata* larvae.

[e]Virus recovery by peroral exposure of second-instar *O. pseudotsugata* larvae.

larvae. The virus does not persist in these fish.

Other investigations have shown nucleopolyhedrosis viruses have no pathological effect on mammalian cell lines (McIntosh and Maramorosch 1973, Ignoffo and Rafajko 1972). Himeno el al. (1967) found that DNA from a nucleopolyhedrosis virus from silkworms was infective in certain mammalian cell lines, but they did not demonstrate infectivity with the intact virion or polyhedra. McIntosh and Shamy (1975) reported that a nucleopolyhedrosis virus from *Autographa californica* became associated with a reptilian cell line, and that certain viral proteins were synthesized by the inoculated cells. They did not observe complete viral replication and no cytopathology was reported.

Wolf (1975) described experiments in which certain amphibian and fish cell lines were exposed to nucleopolyhedrosis viral agents from *O. pseudotsugata*. No cytopathology was found in any of the cell lines tested.

Literature Cited

Berkson, Joseph.
1953. A statistically precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. J. Am. Stat. Assoc. 48(263):565-599.

Fryer, J. L., A. Yusha, and K. S. Pilcher.
1965. The in vitro cultivation of tissues and cells of Pacific salmon and steelhead trout. Ann. N. Y. Acad. Sci. 126:566-586.

Himeno, M., F. Sakai, K. Onodera, H. Nakai, T. Fukada, and Y. Kawade.
1967. Formation of nuclear polyhedral bodies and nuclear polyhedrosis virus of silkworm in mammalian cells infected with viral DNA. Virology 33(3):507-512.

Hughes, K. M., and R. B. Addison.
1970. Two nuclear polyhedrosis viruses of the Douglas-fir tussock moth. J. Invert. Pathol. 16(2): 196-204.

Ignoffo, C. M., and R. R. Rafajko.
1972. In vitro attempts to infect primate cells with the nucleopolyhedrosis virus of *Heliothis*. J. Invert. Pathol. 20(3):321-325.

McIntosh, A. H., and K. Maramorosch.
1973. Retention of insect virus infectivity in mammalian cell cultures. J. N. Y. Entomol. Soc. 81(3):175-182.

McIntosh, A. H., and R. Shamy.
1975. Effects of the nuclear polyhedrosis virus (NPV) of *Autographa californica* on a vertebrate viper cell line. Ann. N. Y. Acad. Sci. 266:327-331.

Martignoni, Mauro E., and Robert J. Scallion.
1961. Preparation and uses of insect hemocyte monolayers in vitro. Biol. Bull. 121(3):507-520.

Wickman, B. E., R. R. Mason, and C. G. Thompson.
1973. Major outbreaks of the Douglas-fir tussock moth in Oregon and California. USDA For. Serv. Gen. Tech. Rep. PNW-5, 18 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oreg.

Wolf, K. E.
1975. Evaluation of the exposure of fish and wildlife to nuclear polyhedrosis and granulosis viruses. *In* Baculoviruses for insect pest control: Safety considerations. Am. Soc. Micro. Publ., p. 109-111.

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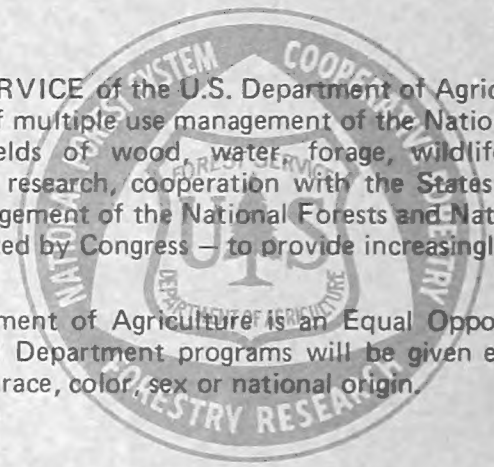

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